



# Impact of miRNA Sequence on miRNA Expression and Correlation between miRNA Expression and Cell Cycle Regulation in Breast Cancer Cells

Zijun Luo<sup>1\*</sup>, Yi Zhao<sup>1</sup>, Robert Azencott<sup>2</sup>

<sup>1</sup> Harbin Institute of Technology, Shenzhen Graduate School, Shenzhen, China, <sup>2</sup> University of Houston, Houston, Texas, United States of America

## Abstract

The miRNAs regulate cell functions by inhibiting expression of proteins. Research on miRNAs had usually focused on identifying targets by base pairing between miRNAs and their targets. Instead of identifying targets, this paper proposed an innovative approach, namely impact significance analysis, to study the correlation between mature sequence, expression across patient samples or time and global function on cell cycle signaling of miRNAs. With three distinct types of data: The Cancer Genome Atlas miRNA expression data for 354 human breast cancer specimens, microarray of 266 miRNAs in mouse Embryonic Stem cells (ESCs), and Reverse Phase Protein Array (RPPA) transfected by 776 miRNAs in MDA-MB-231 cell line, we linked the expression and function of miRNAs by their mature sequence and discovered systematically that the similarity of miRNA expression enhances the similarity of miRNA function, which indicates the miRNA expression can be used as a supplementary factor to predict miRNA function. The results also show that both seed region and 3' portion are associated with miRNA expression levels across human breast cancer specimens and in ESCs; miRNAs with similar seed tend to have similar 3' portion. And we discussed that the impact of 3' portion, including nucleotides 13–16, is not significant for miRNA function. These results provide novel insights to understand the correlation between miRNA sequence, expression and function. They can be applied to improve the prediction algorithm and the impact significance analysis can also be implemented to similar analysis for other small RNAs such as siRNAs.

**Citation:** Luo Z, Zhao Y, Azencott R (2014) Impact of miRNA Sequence on miRNA Expression and Correlation between miRNA Expression and Cell Cycle Regulation in Breast Cancer Cells. PLoS ONE 9(4): e95205. doi:10.1371/journal.pone.0095205

**Editor:** Pranela Rameshwar, Rutgers - New Jersey Medical School, United States of America

**Received:** February 7, 2014; **Accepted:** March 24, 2014; **Published:** April 18, 2014

**Copyright:** © 2014 Luo et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** The authors have no support or funding to report.

**Competing Interests:** The authors have declared that no competing interests exist.

\* E-mail: boluomiduo1@gmail.com

## Introduction

The miRNAs are small non-coding RNAs of roughly 22 nucleotides in length, which can bind with and inhibit protein coding mRNAs through complementary base pairing. A given miRNA can potentially bind and silence hundreds of mRNAs across a number of signaling pathways. By degrading mRNAs and repressing proteins, miRNAs regulate the cell signaling and cell functions.

On the correlation between sequence and function of miRNAs, the central goal of the past research had been to understand how they recognize their target messages. The best characterized features determining the targets of a specific miRNA are the conserved Watson-Crick pairing to the seed (positions 2–7) of the miRNA, which is the so-called "seed pairing rules". [1–5]. Seed rules have been informative [6] to predict targets of miRNAs, especially in combination with microarray or proteomic approaches [7–9].

However, seed pairing rules do not always confer repression of target messages. Efforts have been made to explain that perfect complementarity between miRNA seed and mRNA 3' UTR are neither necessary nor sufficient for all functional miRNA-target interactions [10–14]. False predictions from seed rules could be explained as target transcripts with non-canonical target sites. Chi et al. proposed an alternative mode of miRNA target prediction

[14] and identified functional non-canonical miRNA-mRNA interactions.

To improve the accuracy of predicting miRNA targets, Grimson et al. proposed determinants for targeting beyond seed pairing [12]. The 3' portion of miRNA mature sequence had been identified as one of the additional "context" features that correlate with reduced expression levels of mRNAs. Many target prediction algorithms, including TargetScan and miRanda [2,5,15–17], use base pairing of the 3' portion as an important factor to predict targets though the correlation between 3' portion and targeting is weak.

Besides identification of gene/protein downregulation induced by overly expressed miRNAs, classical microarray analysis also relies on massive application of linear correlation analysis to gene/protein expression profiles [18]. Nonlinear chemical kinetics modeling approach were also proposed to numerically validate potential miRNA-mRNA pairs [19]. Either linear or nonlinear approach implicitly classifies miRNAs with similar time-course expression levels as candidates to have similar regulatory roles. However, it has not been systematically analyzed whether there is a significant correlation between the expression levels and the regulatory function of miRNAs.

In this paper, we studied the correlation between sequence, expression and function of miRNAs by a novel method based on three different sets of data. Interestingly, similar results were

obtained by the same analysis to the TCGA miRNA expression data for 354 breast cancer specimens, miRNA time-course expression data in ESCs and the RPPA transfected by miRNAs in MDA-MB-231 cell line. Our analysis focused only on the "input" (mature sequence) and "output" (miRNA expression and function) without regard to target sites. We linked the expression of miRNAs with their cell cycle regulation and found that miRNA expression (across patient samples or across time) can be considered as a supplementary factor to predict miRNA function. The seed region influence both miRNA expression (across patient samples or across time) and cell cycle signaling independently while the 3' portion is significantly effective for miRNA expression but not for cell cycle regulation. Although the 3' portion had been claimed to have impact on miRNA function[12], we discussed why the 3' portion was rarely found effective. The results provide novel insights to understand correlation between miRNA sequence, expression and function in cells. They can also be applied to improve target prediction algorithms.

## Results

### The Impact of the Seed and the 3' Portion on miRNA Expression and Cell Cycle Regulation

For the expression data of 321 miRNAs across 354 breast cancer specimens, we had calculated the expression distance for each of  $\frac{321 \times 320}{2} = 51360$  miRNA pairs.

We first studied the impact significance of the seed region (nucleotides 1–8) and the 3' portion separately. The set of all miRNA pairs was divided into the following 6 disjoint classes: nucleotides 1–8 identical (CL 1–8); nucleotides 2–8 identical, nucleotide 1 different (CL 2–8); nucleotides 1–7 identical, nucleotide 8 different (CL 1–7); nucleotides 2–7 identical, nucleotides 1,8 different (CL 2–7); nucleotides 3–8 identical, nucleotides 1–2 different (CL 3–8); nucleotides 1,3–8 identical, nucleotide 2 different (CL 1,3–8). Excluding the miRNA pairs with an alignment score of the 3' portion larger than a threshold of 4, we calculated the impact significance of the 6 classes. When we changed the threshold to 6 and to 8, the IS values stay almost the same. Figure 1a shows that CL 1–8, CL 2–8, and CL 1–7 are significantly associated with miRNA expression. The association of CL 1,3–8 is weak ( $p$  value = 0.0018) while CL 2–7 and CL 3–8 have no significant impact.

Similarly, we calculated the IS values of the 3' portion for the miRNA pairs excluding those with identical nucleotides 2–8. Varying thresholds for "high" alignment of the 3' portion (alignment score >4,6,8,10), we can see (Figure 1b) that the 3' portion has a significant influence on miRNA expression.

To examine whether the 3' portion influences miRNA expression when the seeds are identical or highly similar, we searched the impact significance of all possible contiguous segments (3~9 nt) in the 3' portion in each of the six classes. It turned out that segments 11–13, 14–16 and 9–17 are mostly influential (with smallest  $p$  values) for CL 1–8, CL 2–8 and CL 1–7 respectively (Figure 2). And the 3' portion is not effective for CL 2–7, CL 1,3–8 and CL 3–8 at all. Although the 3' portion dissociates with the seed region to impact miRNA expression, we examined the correlation between the alignment scores of the seed region and the 3' portion and found that higher similarity in the seed region tends to be accompanied with higher similarity in the 3' portion (Figure 1c). Classes CL 1–8, CL 2–8 and CL 1–7 tend to be more similar in the 3' portion than CL 2–7, CL 3–8, CL 1,3–8 and the remaining pairs (Figure 1c).

We applied the same analysis to the miRNA time-course expression data in ESCs and the RPPA transfected by 776 miRNAs in MDA-MB-231 cell line and obtained quite similar results (Figures 1d, 1e, 1f, 3a, 3b, 3c). Compared with the results for miRNA expression across TCGA breast cancer specimens, CL 1–7 is not significantly associated with miRNA time-course expression in ESCs. Figure 4 also indicate that the 3' portion is not influential when the seed is highly similar for miRNA time-course expression in ESCs. Figures 3b and 5 show that the impact of the 3' portion is not significant for miRNA function in cell cycle signaling in contrast to its impact on miRNA expression.

### Similarity of miRNA Expression Enhances Similarity of miRNA Cell Cycle Regulation

Based on the above results, it is natural to expect similarity of miRNA expression might be useful to predict similarity of miRNA function. Since the seed, but not the 3' portion, is significantly effective for breast cancer cell regulation, we studied only miRNA pairs with similar seed region and found that indeed similar expression across TCGA breast cancer specimens enhances similarity of cell cycle regulation (Figures 6a). Even when these miRNAs are from different cells (Figure 6b), our statistical analysis still proved that there is a strong correlation between miRNA time-course expression in ESCs and cell cycle regulation in breast cancer cells. Actually, several tested examples of different cell types also support the result, which we discuss in the section below. Therefore, miRNAs with similar seed and expression (across samples) are good candidates to have similar regulatory roles, in other words, miRNA expression (across samples) can be considered as a supplementary determinant to predict the miRNA function.

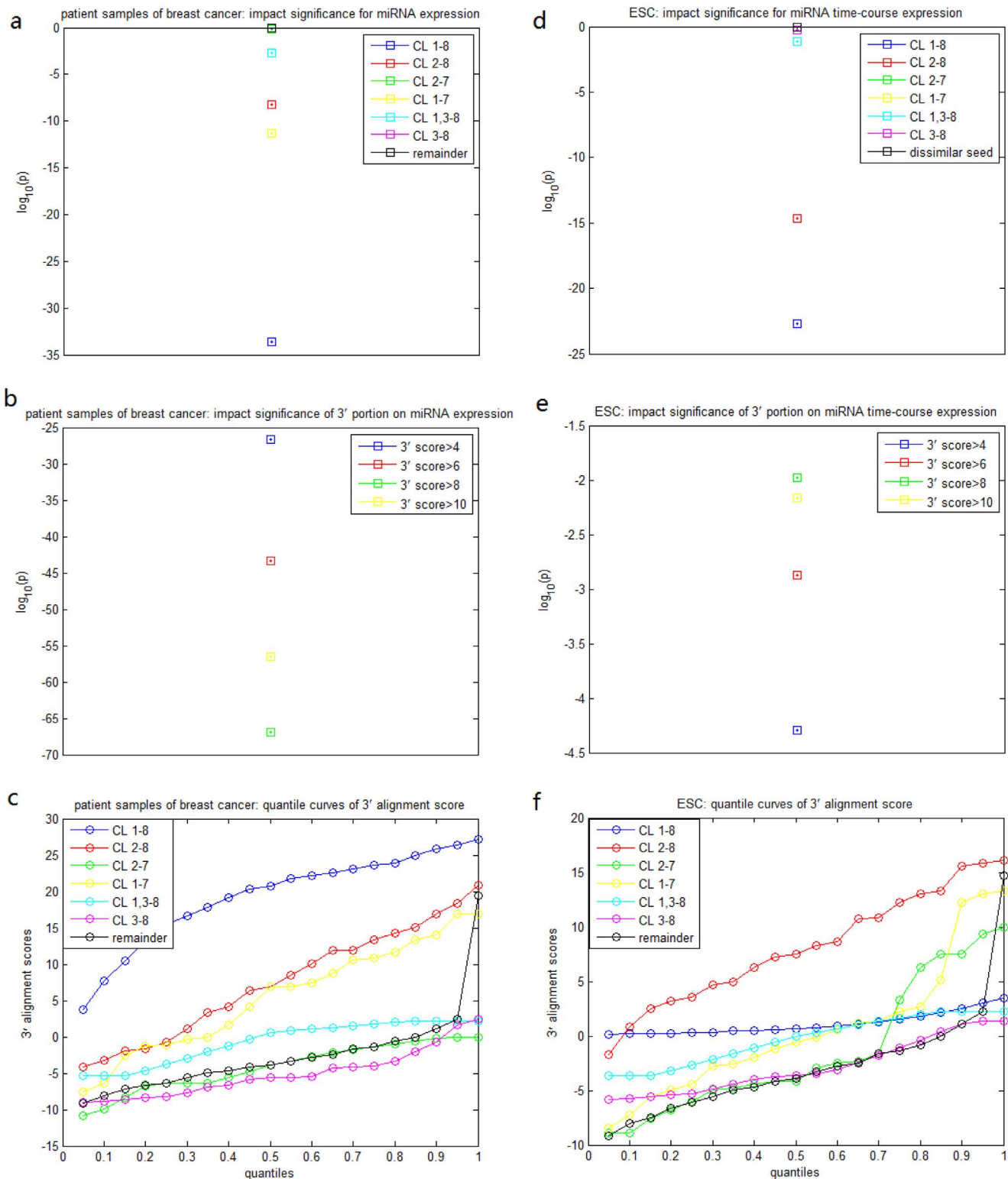
In conclusion, nucleotides 2–8 and 1–8 are influential on both miRNA expression/function while the 3' portion is significantly effective only for expression of miRNAs; the 3' portions tend to be similar when the seed regions are similar; miRNAs with similar mature seed and expression (across samples) are good candidates to have similar regulatory roles.

## Discussion

To analyze the correlation between mature sequence, expression and global function of miRNAs, we defined IS by computing the  $p$ -values of Kolmogorov Smirnov tests applied to compare Euclidean distances of observations of miRNA pairs. This technique allows us to study the differences of global function for distinct miRNAs and the influence of sequence on cell signaling without identifying the targets of these miRNAs. We hence avoided the complexity to determine canonical or noncanonical target sites of UTR and focused on only the miRNA sequence as input and the regulation effect (RPPA) or expression as output. Another advantage of our similarity analysis is that it could be used for clustering miRNAs. For those miRNAs with highly similar seed and expression, it is reasonable to hypothesize that the miRNA cluster have similar regulatory function.

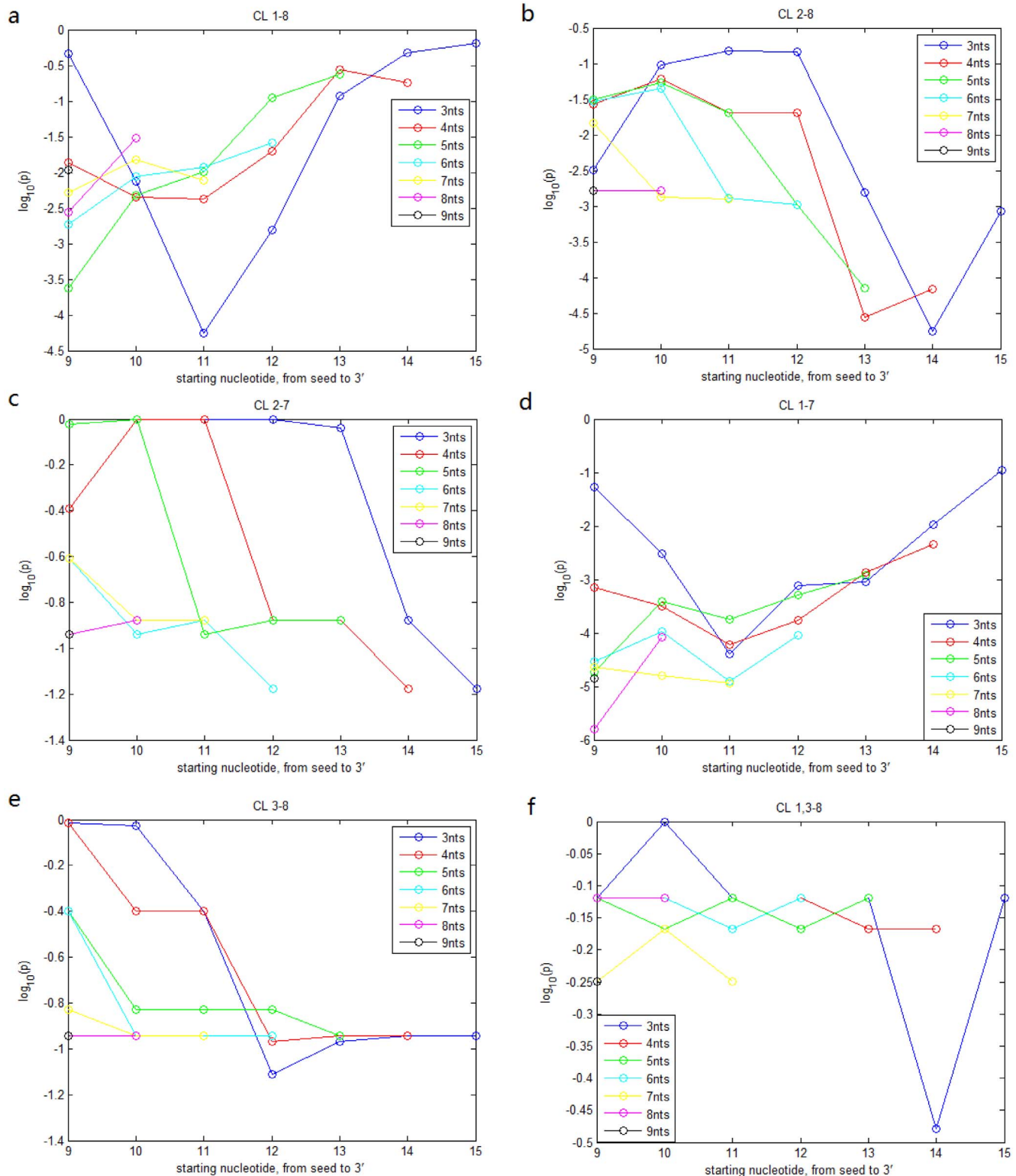
### Literature Support for Association between miRNA Expression and Function

In this paper, we also systematically linked the miRNA expression with their function by alignment score of miRNA sequence. There are some tested examples to support the association between miRNA function, mature sequence, and expression. For instance, miR-103 and miR-107, having very similar mature sequence and expression levels (Figure 6c), are two known miRNAs that have the same roles in regulating insulin



**Figure 1. IS analysis for miRNA expression data.** a. impact significance (IS) of nucleotides 1–8, 2–8, 2–7, 1–7, 3–8, and 1,3–8 for TCGA miRNA expression across breast cancer specimens after excluding miRNA pairs with high alignment score (>4) of the 3' portion. b. IS of the 3' portion for TCGA expression after excluding miRNA pairs with identical nucleotides 2–7 or 3–8. Threshold of similar 3' portion is set to be 4,6,8,10 respectively. c. quantile curves of 3'-alignment scores for 7 groups from the TCGA expression data: the 6 classes and the remaining pairs. Groups with quantile curves above tend to be more similar in the 3' portion than groups with quantile curves below. KS test shows that CL 1–8 2–8, and 1–7 tend to have more similar 3' portion (p values:  $2.3 \times 10^{-34}$ ,  $5.3 \times 10^{-9}$  and  $4.8 \times 10^{-12}$ ). d. e. f. same analysis as a. b. c. for miRNA time-course expression in ESCs.

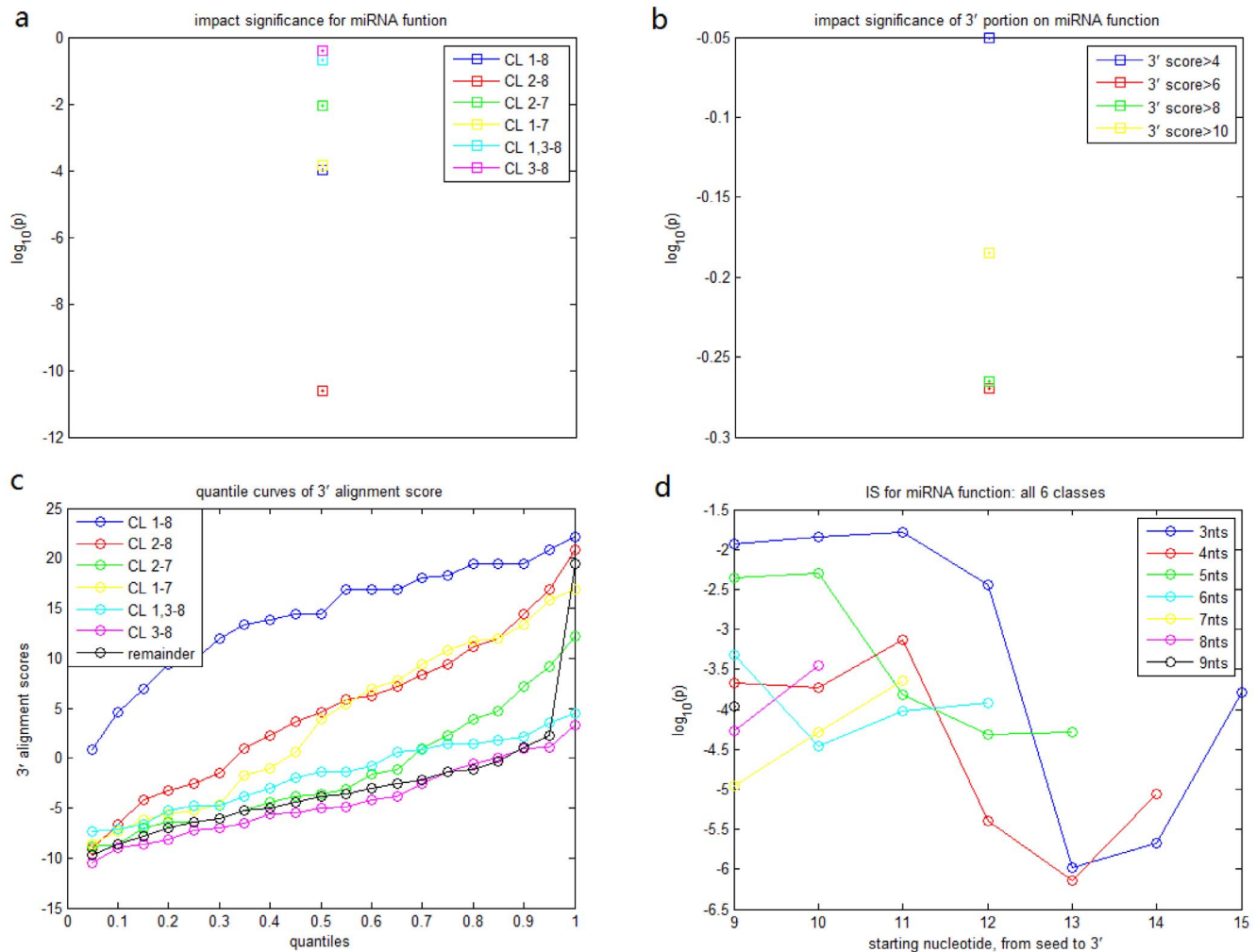
doi:10.1371/journal.pone.0095205.g001



**Figure 2. IS values of the searched contiguous segments of the 3' portion for each of the 6 classes from the TCGA expression data.** 3nts represents 3 contiguous nucleotides, 4nts 4 contiguous nucleotides, and so forth. The x-axis represents the starting position of the segments. doi:10.1371/journal.pone.0095205.g002

sensitivity and promoting metastasis of colorectal cancer [20,21]; miR-34b and miR-34c, having very similar mature sequence and expression levels (Figure 6c), are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth[22];

let-7a/b/c were also claimed to reduces tumor growth in mouse models of lung cancer [23] and miR-29a/b/c reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B [24] while these two miRNA clusters have very similar



**Figure 3. IS analysis for RPPA data.** a. IS of nucleotides 1–8, 2–8, 2–7, 1–7, 3–8, and 1,3–8 for miRNA cell cycle regulation in breast cancer cells after excluding miRNA pairs with high alignment score ( $>4$ ) of the 3' portion. b. IS of the 3' portion for miRNA cell cycle regulation in breast cancer cells after excluding miRNA pairs with identical nucleotides 2–7 or 3–8. Threshold of similar 3' portion is set to be 4,6,8,10 respectively. c. quantile curves of 3'-alignment scores for 7 groups from the breast cancer RPPA data: CL 1–8, 2–8, 2–7, 1–7, 3–8, 1,3–8 and the remaining pairs. Groups with quantile curves above tend to be more similar in the 3' portion than groups with quantile curves below. KS test shows that CL 1–8, 2–8, 2–7 and 1–7 tend to have more similar 3' portion (p values:  $6.3 \times 10^{-61}$ ,  $1.6 \times 10^{-28}$ ,  $5.6 \times 10^{-3}$ ,  $5 \times 10^{-8}$ ). d. IS values of the searched contiguous segments of the 3' portion for all 6 classes from the breast cancer RPPA data. 3nts represents 3 contiguous nucleotides, 4nts 4 contiguous nucleotides, and so forth. The x-axis represents the starting position of the segments. doi:10.1371/journal.pone.0095205.g003

maturation sequences and expression levels (Figure 6d). Thus, we may infer that the conclusion of the paper also holds for expression data and RPPA transfected by miRNAs in different types of cells.

### Noneffectiveness of the 3' Portion, Especially Segment 13–16 for miRNA Cell Cycle Regulation

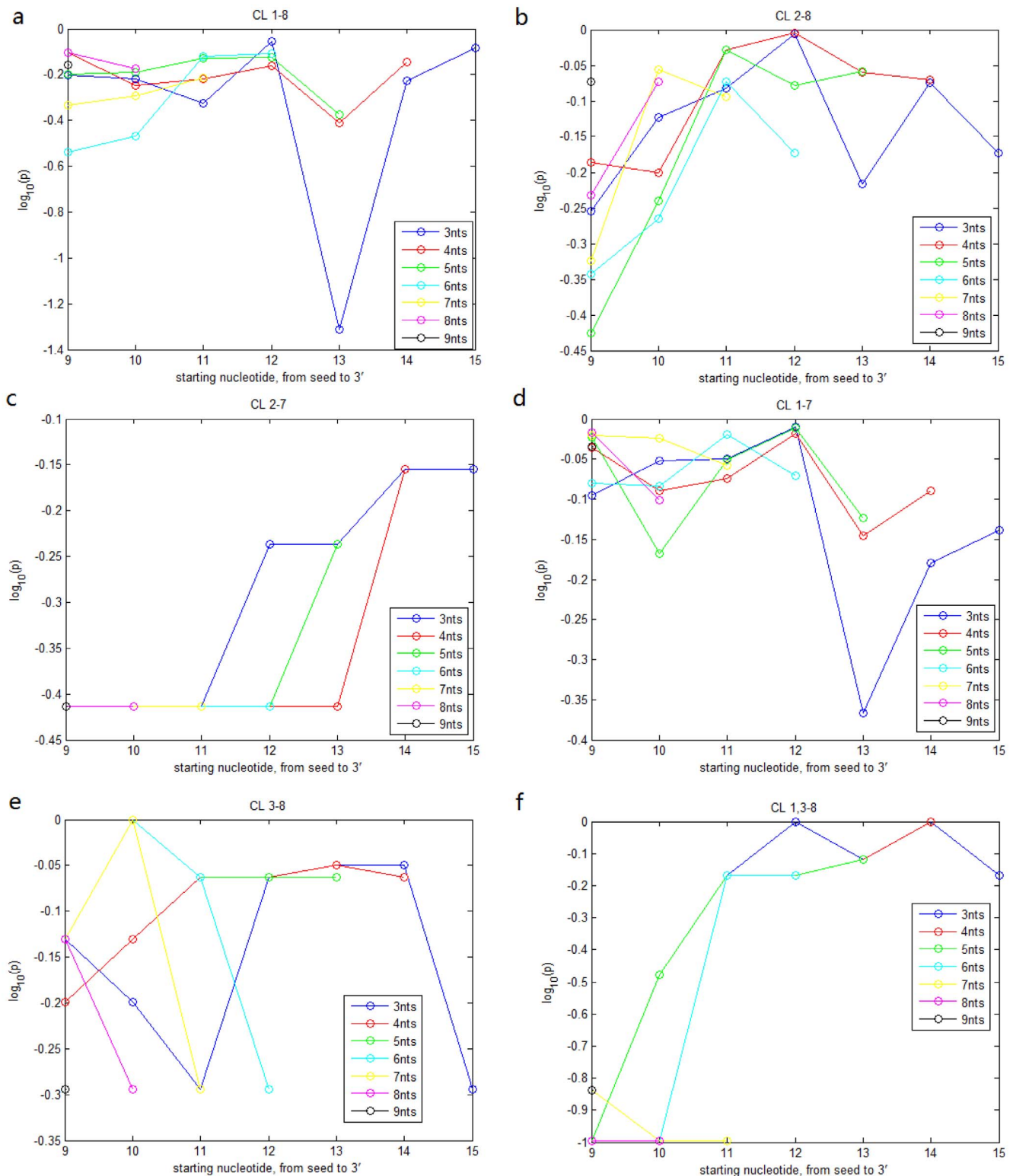
If we consider the impact significance of CL 1–8 is corresponding to the downregulation of 8mer match of seed region; CL 2–8 to 7mer-m8 match; CL 1–7 to 7mer-A1 match; CL 2–7 to 6mer match; then our results about the impact of the seed region for miRNA function are quite consistent with those in [12].

Although Grimson et al. [12] revealed that the 3' portion, especially nucleotides 13–16, enhance the repression of canonical 7mer or mismatched seed sites, our results indicate that the impact of 3' portion is not significant for miRNA function. Systematic

examination of site conservation indicates that mismatched seed sites with 3'-compensatory pairing are only rarely under selective pressure to be conserved [5]. So the statistical difference of results between [12] and our paper could be well explained by the difference in size of the studied data (only 11 miRNAs and microarray data were studied in [12], while RPPA transfected by 776 miRNAs in this paper). To explore deeper the impact of the 3' portion, we examined the miRNA pairs with identical nucleotides 2–7 or 3–8 (including all 6 classes) and found that nucleotides 13–16 seems to most significantly impact the miRNA function (Figures 3d), which is quite consistent with the results of [12].

The above about the impact of 3' portion seems contradictory to our previous results (Figure 5). In fact, CL 1–8, 2–8 and 1–7, having more similar 3' portion (Figure 3c), are more strongly associated with similar miRNA function than CL 2–7, 1,3–8 and 3–8. Consequently the IS analysis shows that the 3' portion (especially nucleotides 13–16) is significantly influential among all

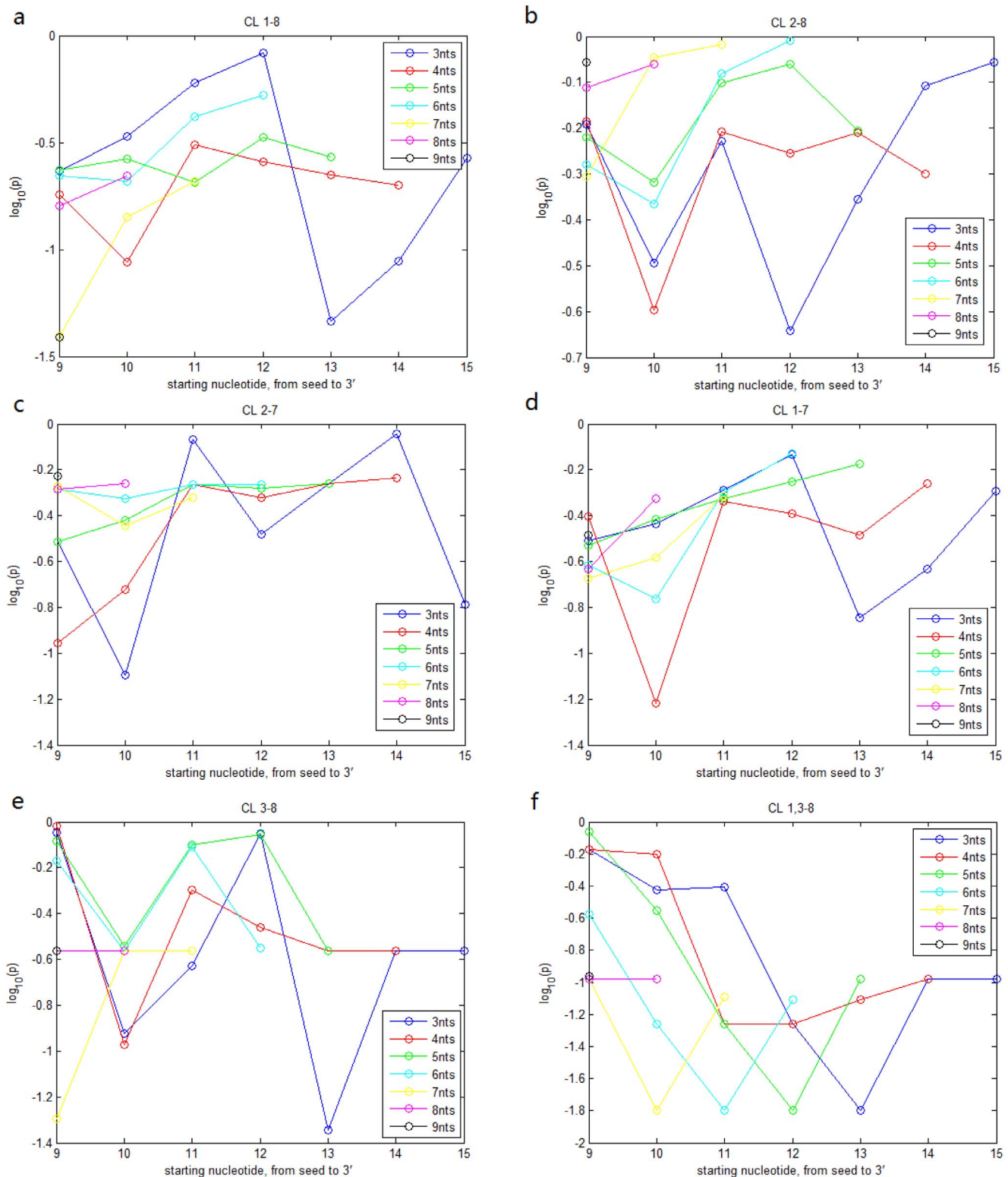




**Figure 4. IS values of the searched contiguous segments of the 3' portion for each of the 6 classes from the expression data of ESCs.** 3nts represents 3 contiguous nucleotides, 4nts 4 contiguous nucleotides, and so forth. The x-axis represents the starting position of the segments. doi:10.1371/journal.pone.0095205.g004

miRNA pairs in our 6 classes. Therefore, the 3' portion seems to be effective because it has a strong association with the seed region, which apparently impacts the miRNA function.

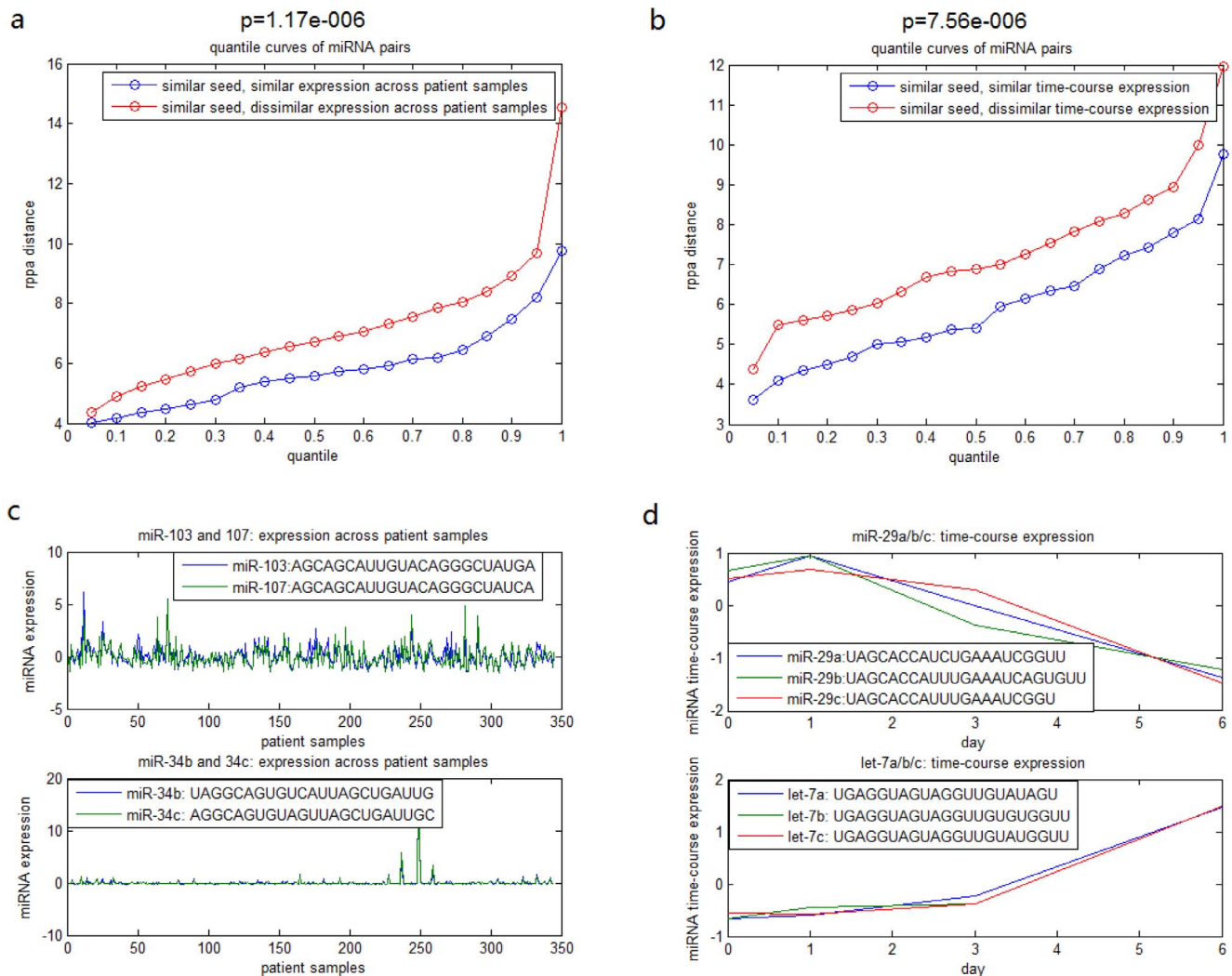
Since our results show that the 3' portion is not effective when the seeds are highly similar, it may be unreasonable to assume that the seed and the 3' region have synergistic influence for the prediction of targets. This remark applies to miRanda algorithm



**Figure 5. IS values of the searched contiguous segments of the 3' portion for each of the 6 classes from the RPPA data.** 3nts represents 3 contiguous nucleotides, 4nts 4 contiguous nucleotides, and so forth. The x-axis represents the starting position of the segments. doi:10.1371/journal.pone.0095205.g005

for miRNA target prediction that uses a weighted sum of match and mismatch scores for base pairs, which is a linear model and implies that the seed and 3' region act synergistically.

In summary, our results provide novel insights on the impact and correlation between the seed, the 3' portion, miRNA expression and function. It is proposed that miRNA expression



**Figure 6. Correlation between miRNA expression and function.** a. quantile curves of two groups for TCGA expression data. group 1: miRNA pairs of similar seed (seed alignment score  $> 5$ ) and similar expression (expression distance  $< 5$ ), group 2: miRNA pairs of similar seed (seed alignment score  $> 5$ ) and dissimilar expression distance (expression distance  $> 25$ ). KS test shows that group 1 tend to have more similar cell cycle regulation than group 2 (p value:  $1.17 \times 10^{-6}$ ). b. quantile curves of two groups for expression data of ESCs. group 1: miRNA pairs of similar seed (seed alignment score  $> 5$ ) and similar expression (expression distance  $< 0.5$ ), group 2: miRNA pairs of similar seed (seed alignment score  $> 5$ ) and dissimilar expression distance (expression distance  $> 3$ ). KS test shows that group 1 tend to have more similar cell cycle regulation than group 2 (p value:  $7.56 \times 10^{-6}$ ). c. expression levels across breast cancer specimens and mature sequences of two miRNA pairs. d. time-course expression levels of ESCs and mature sequences of two miRNA pairs.  
doi:10.1371/journal.pone.0095205.g006

(across samples) can be used as a supplementary factor to predict the miRNA function. These results are also useful to improve target prediction models and algorithms. Our approach can handle large proteomic or genomic data easily and study the global function of miRNAs instead of their targets. It is a novel and easy to use technique to study miRNA expression/function and it could also be possibly applied to similar analysis for other small RNAs such as siRNAs.

## Materials and Methods

### Data Description

The expression profiles of miRNAs across 354 patient samples of breast invasive carcinoma are publicly available on The Cancer Genome Atlas (TCGA: <https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm>). Level 3 miRNA expression data for 354

breast cancer specimens profiled using Illumina GAIIX were downloaded from the TCGA data portal (select a disease: breast invasive carcinoma, data type: miRNASeq, data level: level 3). Mature sequences of 321 individual miRNAs in the list of the paper were collected from miRBase ([www.mirbase.org](http://www.mirbase.org)).

The microarray data of mouse ESCs undergoing RA-induced differentiation, are also publicly accessible from the paper by Gu et al. [18] (see table S1 for the normalized data). The miRNAs microarray was provided by LC Science Inc. Expression levels were recorded for 266 well characterized miRNAs on days 0, 1, 3, 6, based on 8 probe replicates for miRNAs (mmu-miRs).

The RPPA data transfected by miRNAs are publicly accessible online from in the paper by Uhlmann et al. 2011 [25]. Mature sequences of 776 individual miRNA mimics used in our paper were collected from miRBase. Normalized signal intensities of 26 proteins in cell cycle pathway were recorded in each well



transfected by miRNAs in MDA-MB-231 cell line (see table S2 for the normalized RPPA data collected from table S5 in [25] and the mature sequence from miRBase).

## Data Pretreatment

For the TCGA expression data, we eliminated all miRNA profiles with extremely low expression (less than 20) in all the samples and thus obtained the profiles of 321 distinct miRNAs. The expression levels across 354 patients for each miRNA had been normalized by its own mean and standard deviation. For the time-course data of miRNAs, the expression levels of each day were averaged for replicates. After taking logarithm of the data, each observation of the time-course expression levels of the 266 miRNAs were normalized by its own mean value and standard deviation.

## Methods

We first defined the RPPA distance of any miRNA pair ( $M_i$ ,  $M_j$ ) to be the Euclidean distance of the RPPA data  $\mathbf{p}_i$  and  $\mathbf{p}_j$ , where  $\mathbf{p}_i$  is the vector  $(p_{i,k})_{k=1,\dots,26}$  and  $p_{i,k}$  represents the normalized expression of  $k^{th}$  protein transfected by miRNA  $M_i$ . Similarly, we defined the expression distance of any miRNA pair ( $M_i$ ,  $M_j$ ) to be the Euclidean distance of the expression data  $\mathbf{m}_i$  and  $\mathbf{m}_j$ , where  $\mathbf{m}_i$  is the vector  $(m_{i,k})_{k=1,\dots,4}$  and  $m_{i,k}$  represents the normalized expression at  $k^{th}$  patient samples or time point of miRNA  $M_i$ .

The alignment score between any miRNA pair ( $M_i$ ,  $M_j$ ) were computed by Needleman-Wunsch algorithm (Matlab function: `nwalign`). Let  $S$  be a subsequence of miRNA mature sequence, such as for instance nucleotides 2–8. For any subsequence  $S$ , we quantify the similarity of two miRNAs by their alignment score  $score(S)$  on sequence  $S$ . Then we can divide all the available miRNA pairs ( $776 \times 775/2 = 300700$  pairs for the RPPA data,  $266 \times 265/2 = 35245$  for the miRNA time-course data) into two groups: let group 1 be the group of miRNA pairs with high

alignment  $score(S)$ , group 2 be the group of miRNA pairs with low alignment  $score(S)$ . We used Kolmogorov-Smirnov (KS) test to compare the distribution of rppa/expression distance for the two groups and got a p value  $K$ . We define "impact significance" (IS) of  $S$ , where  $S$  denotes a given segment of the miRNA mature sequence, by the formula:

$$IS = \log_{10}(K)$$

Note that if  $IS$  is smaller than some threshold such as  $\log_{10}(0.01) \sim -2$ , then it implies that for miRNAs pairs with strong  $S$  similarity, the rppa/expression tend to be similar.

Our approach focused on the similarity of miRNA pairs in sequence, expression and global function, while traditional methods focused on base-pairing between miRNA and target messages, and downregulation effects.

## Supporting Information

**Table S1 miRNA mature sequence and normalized expression at day 0, 1, 3, 6 in mouse ES cells.**

(XLS)

**Table S2 miRNA sequence and RPPA data of MDA-MB-231 cell line transfected by each individual miRNA.**

(XLS)

## Acknowledgments

We would like to thank Dr. P. H. Ram, Dr. G. B. Mills and Dr. Y. Lu in MD Anderson Cancer Center for multiple discussions concerning their RPPA data on miRNA regulating cell signaling.

## Author Contributions

Analyzed the data: ZL. Contributed reagents/materials/analysis tools: ZL YZ RA. Wrote the paper: ZL RA. Collected publicly accessible data: ZL.

## References

- Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB (2003) Prediction of mammalian microRNA targets. *Cell* 115: 787–798.
- Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120: 15–20.
- Brennecke J, Stark A, Russell RB, Cohen SM (2005) Principles of microRNA target recognition. *PLoS Biol* 3: e85.
- Krek A, Grün D, Poy MN, Wolf R, Rosenberg L, et al. (2005) Combinatorial microRNA target predictions. *Nat Genet* 37: 495–500.
- Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. *Cell* 136(2): 215–33.
- Lim LP, Lau NC, Garrett-Engle P, Grimson A, Schelter JM, et al. (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 433: 769–73.
- Back D, Villén J, Shin C, Camargo FD, Gygi SP, et al. (2008) The impact of microRNAs on protein output. *Nature* 455: 64–71.
- Selbach M, Schwanhäusser B, Thierfelder N, Fang Z, Khanin R, et al. (2008) Widespread changes in protein synthesis induced by microRNAs. *Nature* 455: 58–63.
- Mourelatos Z (2008) Small RNAs: The seeds of silence. *Nature* 455: 44–45.
- Ha I, Wightman B, Ruvkun G (1996) A bulged lin-4/lin-14 rna duplex is sufficient for caenorhabditis elegans lin-14 temporal gradient formation. *Genes Dev* 10: 3041–50.
- Vella MC, Choi EY, Lin SY, Reinert K, Slack FJ (2004) The c. elegans microRNA let-7 binds to imperfect let-7 complementary sites from the lin-41 3'UTR. *Genes Dev* 18: 132–7.
- Grimson A, Farh KK, Johnston WK, Garrett-Engle P, Lim LP, et al. (2007) MicroRNA targeting specificity in mammals: Determinants beyond seed pairing. *Molecular Cell* 27(1): 91–105.
- Tay Y, Zhang J, Thomson AM, Lim B, Rigoutsos I (2008) MicroRNAs to nanog, oct4 and sox2 coding portions modulate embryonic stem cell differentiation. *Nature* 455: 1124–1128.
- Chi SW, Hannon GJ, Darnell RB (2012) An alternative mode of microRNA target recognition. *Nat Struct Mol Biol* 19(3): 321–327.
- Betel D, Wilson M, Gabow A, Marks DS, Sander C (2008) The microRNA.org resource: targets and expression. *Nucleic Acids Res* 36(Database Issue): D149–53.
- Friedman RC, Farh KK, Burge CB, Bartel DP (2009) Most mammalian mRNAs are conserved targets of microRNAs. *Genome Research* 19: 92–105.
- Garcia DM, Back D, Shin C, Bell GW, Grimson A, et al. (2011) Weak seed-pairing stability and high target-site abundance decrease the proficiency of lsc-6 and other miRNAs. *Nat Struct Mol Biol* 18: 1139–1146.
- Gu P, Reid JG, Gao X, Shaw CA, Creighton C, et al. (2008) Novel miRNA candidates and miRNA pairs in ES cells. *PLoS ONE* 3(7): e2548.
- Luo Z, Xu X, Gu P, Leonard D, Gunaratne P, et al. (2011) Regulatory circuits of miRNAs in ES cell differentiation: a chemical kinetics modeling approach. *PLoS One* 6(10): e23263.
- Trajkovski M, Hausser J, Soutschek J, Bhat B, Akin A, et al. (2011) MicroRNAs 103 and 107 regulate insulin sensitivity. *Nature* 474(7353): 649–53.
- Chen HY, Lin YM, Chung HC, Lang YD, Lin CJ, et al. (2012) mir-103/107 promote metastasis of colorectal cancer by targeting the metastasis suppressors *dapk* and *klf4*. *Cancer Res* 72(14): 3631–41.
- Corney DC, Flesken-Nikitin A, Godwin AK, Wang W, Nikitin AY (2007) MicroRNA-34b and microRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. *Cancer Res* 67: 8433.
- Esquela-Kerscher TPA, Wiggins JF, Patrawala L, Cheng A, Ford L, et al. (2008) The let-7 microRNA reduces tumor growth in mouse models of lung cancer. *Cell Cycle* 7(6): 759–64.
- Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, et al. (2007) MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3a and 3b. *Proc Natl Acad Sci U S A* 104(40): 15805C15810.
- Uhlmann S, Mannsperger H, Zhang JD, Á HE, Schmidt C, et al. (2011) Global microRNA level regulation of egfr-driven cell-cycle protein network in breast cancer. *Nat Molecular Systems Biology* 8: 570.